Medical Science

Biomarkers in Rheumatoid Arthritis. Review of the current state of knowledge

Adrian Kruszewski[1*], **Natalia Paduszyńska**[1], **Anna Dąbrowska**[1], **Barbara Wawrzyńska**[2], **Karolina Strus**[3], **Roksana Zdunek**[4], **Magdalena Madera**[5]

To Cite:
Kruszewski A, Paduszyńska N, Dąbrowska A, Wawrzyńska B, Strus K, Zdunek R, Madera M. Biomarkers in Rheumatoid Arthritis. Review of the current state of knowledge. *Medical Science* 2024; 28: e84ms3389 doi: https://doi.org/10.54905/disssi.v28i149.e84ms3389

Authors' Affiliation:
[1]Dr Anna Gostynska Wolski Hospital, Independent Public Health Care Institution, Marcina Kasprzaka 17, 01-211 Warsaw, Poland
[2]MEDIQ Clinic, Józefa Piłsudskiego 20, 05-120 Legionowo, Poland
[3]Independent Public Specialist Western Hospital named after St John Paul II, Daleka 11 05-825 Grodzisk Mazowiecki, Poland
[4]Miedzylesie Specialist Hospital in Warsaw, Bursztynowa 2, 04-749 Warsaw, Poland
[5]The University Clinical Hospital named after F. Chopin in Rzeszów, Fryderyka Szopena 2, 35-055 Rzeszów, Poland

[*]**Corresponding Author**
Dr Anna Gostynska Wolski Hospital, Independent Public Health Care Institution, Marcina Kasprzaka 17, 01-211 Warsaw, Poland
Email: adrkru7@gmail.com

Contact List

Adrian Kruszewski	adrkru7@gmail.com
Natalia Paduszyńska	natalia_paduszynska@onet.eu
Anna Dąbrowska	annaalicjadabrowska06@gmail.com
Barbara Wawrzyńska	b.konerberger@gmail.com
Karolina Strus	karolina.strus2@gmail.com
Roksana Zdunek	zdunekro@gmail.com
Magdalena Madera	m.madera30@gmail.com

ORCID List

Adrian Kruszewski	0009-0003-6077-4948
Natalia Paduszyńska	0000-0001-9953-662X
Anna Dąbrowska	0009-0003-2289-5909
Barbara Wawrzyńska	0009-0006-7504-304X
Karolina Strus	0009-0002-2722-5210
Roksana Zdunek	0009-0009-3723-375X
Magdalena Madera	0009-0004-9668-2436

Peer-Review History
Received: 03 May 2024
Reviewed & Revised: 07/May/2024 to 15/July/2024
Accepted: 19 July 2024
Published: 29 July 2024

Peer-review Method
External peer-review was done through double-blind method.

Medical Science
pISSN 2321–7359; eISSN 2321–7367

DISCOVERY SCIENTIFIC SOCIETY

ABSTRACT

Rheumatic arthritis (RA) is a complex autoimmune disease characterized by chronic inflammation and joint destruction. Recent progress in the study of RA has been centered around pinpointing biomarkers that can assist in early detection, monitoring the disease, and tailoring treatment plans to the individual. While conventional biomarkers like rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) have played a significant role in RA diagnosis, their precision and accuracy exhibit variability. Novel biomarkers, such as microRNAs (miRNAs/miR) and specific cytokines like interleukin-6 (IL-6), can provide deeper insights into disease mechanisms and monitor therapeutic responses. Despite advancements in biomarkers research, challenges remain in effectively standardizing and integrating biomarker assays into clinical practice. Future directions in biomarker research promise to enhance precision medicine approaches and improve outcomes for RA patients. This study aims to discuss the current landscape of biomarkers in RA, focusing on critical indicators such as RF, ACPA, C-reactive protein (CRP), and other novel markers.

Keywords: Rheumatoid arthritis; biomarker; antibodies; RF; microRNA

1. INTRODUCTION

RA is a persistent inflammatory condition with an estimated prevalence ranging from 0.5% to 1% of the population (Smolen et al., 2016). This disease causes alterations in the synovial tissue of joints, cartilage, and bone and can also lead to various extra-articular manifestations such as rheumatoid nodules, pulmonary complications, and vasculitis (Scherer et al., 2020; Smolen et al., 2016). RA typically affects a few joints initially, but as it progresses, multiple joints become involved (Lin et al., 2020). RA negatively impacts the overall quality of life, leading to challenges in both physical and mental well-being (Matcham et al., 2014). The exact cause of RA is still not fully understood, but factors contributing

to its development include genetic predisposition and autoimmune processes (Scherer et al., 2020).

Individuals with RA often experience tender, swollen joints, morning stiffness in joints, as well as general symptoms like fatigue and flu-like signs. The disease is suspected based on the patient's symptoms, physical examination results, risk factors evaluation, family history, and other additional tests. Physicians should refer to the 2010 American College of Rheumatology-European League against Rheumatism (ACR-EULAR) diagnostic criteria (Lin et al., 2020). Patients with RA could be divided into two groups based on RF and ACPAs presence: Seropositive for those with the antibodies and seronegative for those without (Perera et al., 2024). The treatment includes using non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, and disease-modifying antirheumatic drugs (DMARDs) (Smolen et al., 2016). Non-pharmacological approaches such as physiotherapy, lifestyle adjustments, and surgical interventions to address affected joint and bone areas are also part of the treatment plan (Lin et al., 2020).

The initial choice for pharmacological treatment should consist of traditional synthetic DMARDs, particularly methotrexate, in combination with low-dose glucocorticoids (Smolen et al., 2016). It is essential to recognize that while glucocorticoids provide quick relief from symptoms and can modify the progression of the disease, their prolonged use is restricted due to severe side effects (Lin et al., 2020; Smolen et al., 2016). Although NSAIDs can help alleviate pain and stiffness, they do not impede the advancement of the disease. Despite the treatment options, not all patients achieve complete remission or significantly reduce disease activity (Lin et al., 2020). It is essential to monitor disease activity in individuals undergoing management (Smolen et al., 2016). Assessment of biomarkers is crucial in the diagnostic process (Lin et al., 2020).

Aim

This article presents the latest data on biomarkers in RA and their characteristics and significance. The study aims to provide clinicians with a comprehensive overview of biomarkers, translating to improved diagnostic and therapeutic strategies for RA patients. By synthesizing recent advancements, this article highlights the potential of biomarkers to enhance personalized medicine and improve patient outcomes in RA.

2. MATERIAL AND METHODS

This review analyzed materials available in two medical databases - Pubmed and Google Scholar. To find relevant articles as keywords, we used "rheumatoid arthritis" or "marker" or "antibodies" or "microRNA". The databases were searched on 30th April 2024. The inclusion criteria were the year the study was published and the type of article. Research published more than ten years ago was excluded. Under consideration, we took meta-analyses, systematic reviews, reviews, randomized controlled trials, and clinical trials. We rejected papers that did not have the full text available. The initial screening procedure included assessing articles based on their titles and whether they were written entirely in English. Then, based on the abstracts, we selected articles for full-text reading. The quality and significance of the studies determined the ultimate incorporation in our analysis.

3. RESULTS AND DISCUSSION

Inflammatory reactants

CRP is an acute-phase reactant, showing elevated levels in infectious and noninfectious conditions and during inflammatory states (Shapiro, 2021). The synthesis of CRP occurs in hepatocytes when stimulated by interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-17 (IL-17), and other cytokines (Imas et al., 2020). Research suggests that smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes also play a role in CRP production. An average circulating CRP level is typically below ten mg/L, but in patients with RA, CRP levels tend to remain consistently elevated (Pope and Choy, 2021). It is important to note that the serum concentration of CRP varies individually, and factors such as age, sex, and race could influence its value (Shapiro, 2021).

Studies have shown a correlation between serum and synovial fluid CRP concentration, IL-6 levels, and disease activity in individuals with RA (Pope and Choy, 2021). CRP is included in the 2010 ACR/EULAR Classification Criteria for RA and is also used to evaluate disease activity in the Disease Activity Score in 28 joints (DAS28-CRP) (Imas et al., 2020; Shapiro, 2021). The elevation of CRP levels has been associated with the severity of the disease, changes in the synovium's histology, progression seen in radiological imaging, and the intensity of symptoms (Lin et al., 2020). However, approximately 40% of RA cases may have average CRP serum concentrations (Shapiro, 2021).

In addition to its role as an inflammatory reactant, CRP acts as an immune regulator by activating the classical complement pathway, stimulating neutrophils, and enhancing their phagocytic activity. CRP binds to immunoglobulin Fc gamma receptors (FcγR), producing proinflammatory cytokines and promoting the survival and proliferation of macrophages (Lin et al., 2020; Pope and Choy, 2021). There is evidence pointing to the potential role of CRP in contributing to bone destruction in individuals with RA. Literature data indicates that CRP induces the expression of receptor activator of the nuclear factor-κB ligand (RANKL), leading to bone resorption. Additionally, CRP stimulates RANKL expression in blood monocytes and promotes osteoclast differentiation independently of RANKL (Pope and Choy, 2021).

Among the laboratory tests used to detect the acute phase response is the erythrocyte sedimentation rate (ESR). Although ESR currently has limited diagnostic value, it is still utilized in rheumatic diseases (Lapić et al., 2020). The biomarker mentioned is cited in the 2010 ACR/EULAR Classification Criteria for RA and could be a factor in assessing disease severity through the Disease Activity Score in 28 joints (DAS28-ESR) (Shapiro, 2021). When the plasma contains high levels of positively charged proteins like fibrinogen or immunoglobulins, the electrical repulsion decreases, causing erythrocytes to clump together faster, leading to expedited blood cell sedimentation. Literature suggests an increased fibrinogen level is the primary reason for elevated ESR during inflammatory phases (Hale et al., 2019).

Elevated ESR is not a specific indicator for RA, as it may be linked to other physiological conditions like pregnancy or pathological conditions such as anemia or multiple myeloma (Lin et al., 2020). It is also important to note that not all RA patients exhibit elevated ESR (Shapiro, 2021). In a group of patients with RA, elevated levels of IL-6 can be detected in the affected joints' bloodstream and the synovial fluid (Pandolfi et al., 2020). IL-6, a cytokine with pleiotropic effects, is produced by T lymphocytes, B lymphocytes, monocytes, and activated synovial fibroblasts (Favalli, 2020; Takeuchi et al., 2021). IL-6 influences osteoclast activity by increasing RANKL production and enhancing bone resorption (Favalli, 2020). It also plays a crucial role in differentiating Th17 cells, with RA patients exhibiting more of these cells in their blood than healthy individuals.

Furthermore, IL-6 enhances the production of immunoglobulins G, M, and A (IgG, IgM, and IgA) and promotes acute-phase protein synthesis by liver cells (Takeuchi et al., 2021). Studies indicate a correlation between IL-6 levels and the severity of RA, with successful treatment using DMARDS or tumor necrosis factor (TNF) inhibitors resulting in a reduction in serum IL-6 concentrations. Lowering IL-6 levels in the first year of therapy is a prognostic indicator of improved clinical outcomes (Yoshida and Tanaka, 2014). Notably, due to its involvement in the disease's pathogenesis, the IL-6 receptor (IL-6R) blockade is utilized in treatment (Favalli, 2020).

Antibodies

Initially, autoantibodies identified in patients with RA were RF (Van-Delft and Huizinga, 2020). RF refers to autoantibodies of IgM, IgG, and IgA classes that target the Fc-fragment of IgG (Sokolova et al., 2022). Despite its name, RF is not exclusive to RA (Wu et al., 2021). The specificity and sensitivity of this biomarker are presented in (Table 1). The presence of this marker in healthy individuals varies by population. Literature suggests its prevalence is approximately 1.3–4% in Caucasians and can be as high as 30% in certain North American Indian groups (Van-Delft and Huizinga, 2020). Furthermore, the presence of this biomarker is associated with age, as more than twenty-five percent of individuals over the age of 85 exhibit this indicator. RF can emerge as a response post-vaccination or secondary immune response to combat microorganisms (Wu et al., 2021).

This biomarker may also be present in patients with other rheumatological conditions like systemic lupus erythematosus or Sjogren's disease (Van-Delft and Huizinga, 2020; Wu et al., 2021). Moreover, it's important to note that conditions unrelated to rheumatology, such as C-virus infection, cryoglobulinemia, and cancer, have been linked to RF. RF is referenced in the currently accepted diagnostic criteria (Sokolova et al., 2022). Researchers propose that variability in the concentration of this biomarker does not align with disease progression. As a result, continuous monitoring of RF levels is not recommended (Shapiro, 2021). Certain studies indicate that RF of the IgA class may have a more significant impact than other immunoglobulins. In addition, it has been found that the IgA biomarker was associated with erosive disease. (Sokolova et al., 2022).

Table 1 Specificity and sensitivity of antibodies used in the diagnosis of RA

Biomarker	Specificity	Sensitivity	Authors, year
Rheumatoid factor (RF)	85%	60 - 90%	Sokolova et al., 2022
Anti-citrullinated protein antibodies (ACPA)	86–99%	60 - 78%	Sokolova et al., 2022
Anti-carbamylated protein (anti-CarP) antibodies	89%	44%	Kwon and Ju, 2021

Like RF, ACPAs are part of the ACR/EULAR diagnostic criteria. ACPA is considered a more valuable biomarker for RA than RF (Iyengar et al., 2021). ACPAs represent a diverse group of antibodies that can belong to various classes, such as IgG, IgA, and IgM (Catrina et al., 2021; Kurowska et al., 2017). As the name implies, they target citrullinated peptide molecules (Iyengar et al., 2021). Citrullinated peptides are produced during post-translational modifications of proteins due to the deimination of arginine residue by the intracellular enzyme peptidyl arginine deiminase (PAD), resulting in citrulline, a non-essential amino acid. These newly formed epitopes trigger an immune response (Catrina et al., 2021; Kurowska et al., 2017; Shapiro, 2021).

Factors like genetics and environmental influences, including smoking, play a role in developing this immune response. However, the precise mechanism behind ACPA development remains unclear (Iyengar et al., 2021). Around 1% to 3% of people in the general population have detectable ACPAs (Catrina et al., 2021). Table 1 presents the sensitivity and specificity of ACPA for diagnosing RA. Individuals in the general population with detected ACPAs are more likely to develop RA, experience bone loss, and suffer from joint pain even before the disease becomes apparent (Catrina et al., 2021). Notably, ACPAs could also signal the potential development of conditions like Sjögren's syndrome or systemic lupus (Iyengar et al., 2021). Antibody production may commence many years before the onset of the disease (Kurowska et al., 2017).

Additionally, the research literature suggests that joint abnormalities visible on radiographs manifest at an earlier disease stage in ACPA-positive patients (Iyengar et al., 2021). A critical effect of ACPA interaction with citrullinated proteins is the formation of immune complexes (IC) capable of triggering the complement system activation, which releases chemotactic factors like C3a and C5a. This activation can also recruit immune cells (Toes and Pisetsky, 2019). ACPA can activate the complement system via both classical and alternative pathways, which, through an IC-mediated mechanism, promotes macrophage activation and enhances the production of pro-inflammatory cytokines (Catrina et al., 2021).

In laboratory studies, researchers have shown that these antibodies, through Fcγ receptors, stimulate macrophages to synthesize TNF-α (Sokolova et al., 2022). ACPA may also boost osteoclast activity and bone resorption by activating osteoclasts through the receptor activator of nuclear factor-κB (RANK) and RANKL pathways (Iyengar et al., 2021). ACPA-positive RA patients exhibited elevated bone resorption markers such as tartrate-resistant acid phosphatase 5b (TRAP5), cathepsin K, and C-terminal telopeptide of type I collagen (CTX-I) in their bone marrow. The relationship between these antibodies and pain remains a research interest (Sokolova et al., 2022). A mouse model study found that injecting polyclonal ACPA led to pain-like behavior in animals, even without signs of inflammation (Catrina et al., 2021).

However, a survey of RA-diagnosed individuals revealed no direct correlation between ACPA presence and pain at diagnosis (Sokolova et al., 2022). Among individuals diagnosed with RA, researchers have discovered the existence of anti-carbamylated protein (anti-CarP) antibodies (Van-Delft and Huizinga, 2020). Like citrullination, carbamylation represents a post-translational modification of molecules (Carubbi et al., 2019). During carbamylation, lysine residues change in the presence of cyanide, resulting in homo-citrulline formation (Carubbi et al., 2019; Ricchiuti et al., 2022). Homo-citrulline differs from citrulline by having an additional CH2 in its side chain (Ricchiuti et al., 2022). Cyanate, present in body fluids, is maintained at equilibrium with urea levels (Van-Delft and Huizinga, 2020).

In healthy individuals, cyanate levels are typically insufficient to induce permanent carbamylation. However, elevated cyanate levels are observed during conditions like uremia, inflammation, and exposure to cigarette smoke (Carubbi et al., 2019). Homo-citrulline enhances immunogenicity, triggering the production of anti-CarP antibodies in the body (Kwon and Ju, 2021). Research data suggests that anti-CarP antibodies can be detected in up to 45% of RA cases (Wu et al., 2021). Anti-CarP antibodies are independent of ACPA or RF (Kwon and Ju, 2021; Ricchiuti et al., 2022). Notably, 6%–30% of seronegative RA patients are estimated to have anti-CarP

antibodies (Carubbi et al., 2019). These antibodies may also be present in healthy individuals and other rheumatic disorders (Wu et al., 2021).

The sensitivity of anti-CarP antibodies in diagnosing RA is relatively low, while the specificity is higher (Kwon and Ju, 2021). Their values are in (Table 1). According to research, anti-CarP antibodies may be identifiable many years before the emergence of RA, with their concentrations progressively rising before the onset of the disease (Van-Delft and Huizinga, 2020). Additionally, anti-CarP antibodies have been linked to radiological disease progression irrespective of ACPA presence (Ricchiuti et al., 2022). Detecting these antibodies before the onset of RA serves as a predictive factor for erosive disease (Carubbi et al., 2019). In addition, people with RA who have positive test results for anti-CarP antibodies showed higher mortality rates (Ricchiuti et al., 2022).

MicroRNA

MicroRNA comprises a single-stranded, approximately 20–22 nucleotides long, non-coding RNA molecule (Kmiołek and Paradowska-Gorycka, 2022). It orchestrates the expression of numerous genes post-translationally (Kmiołek and Paradowska-Gorycka, 2022; Zhang et al., 2023). A substantial fraction of the human genome, roughly one-third, is believed to be influenced by miRNA (Kmiołek and Paradowska-Gorycka, 2022). Typically, genes that encode miRNAs are located within intronic sequences (Peng et al., 2023). The biogenesis of miRNA initiates during DNA transcription, giving rise to a lengthy primary pri-miRNA. Subsequently, the pri-miRNA transcripts undergo enzymatic modifications to form pre-miRNA. Further processing by enzymes transforms pre-miRNA into a short single-stranded miRNA, concluding with the modification of double-stranded miRNA (Zhang et al., 2023).

The generation of miRNAs takes place intracellularly, where miRNA exerts its effects. Nevertheless, there is a potential to release miRNA into various bodily fluids (Peng et al., 2023). Existing literature indicates that miRNA expression is altered in multiple tissues and cells of individuals with RA (Doghish et al., 2023). Dysregulated miRNA profiles have been observed in RA patients' serum, synovial fluid, and peripheral blood monocytes (PBMC) (Zhang et al., 2023). Furthermore, researchers are currently investigating miRNA as a potential new biomarker (Doghish et al., 2023). It is important to note that not all individuals with RA possess antibodies, underscoring the need to explore new markers (Zhang et al., 2023). Research findings indicate a correlation between the progression of rheumatoid arthritis (RA) and miRNA levels.

For instance, plasma miR-16, miR-146a, miR-155, and miR-223 levels are lower in patients at the early stages of RA than those with more advanced disease. Existing literature suggests that miR-146a and miR-155 play crucial roles in the early detection of RA (Kmiołek and Paradowska-Gorycka, 2022). Studies indicate that pro-inflammatory cytokines play a role in the increased expression of miR-146a, a microRNA that plays a crucial role in controlling and regulating functions of both innate and adaptive immune cells and influencing their differentiation. Studies indicate that individuals with RA have elevated levels of miR-146a in monocytes, macrophages, B cells, T cells, and CD4+ cells that produce IL-17 (Bae and Lee, 2018). Studies suggest that described as a "double-edged sword" in the literature, miR-146a plays a role in sustaining inflammation by preventing T-cell apoptosis (Bae and Lee, 2018; Kmiołek and Paradowska-Gorycka, 2022).

Researchers believe that this microRNA restrains proliferation, impacts metabolism, and diminishes the osteoclastogenic potential of fibroblast-like synoviocytes (FLS) in RA patients. It forms part of a negative feedback loop in macrophages, decreasing the synthesis of inflammatory factors (Peng et al., 2023). The deficiency of miR-146a in T regulatory cells could contribute to a breach of immunological tolerance. Elevated levels of miR-146a have been associated with inhibiting osteoclastogenesis, thereby suppressing bone destruction (Kmiołek and Paradowska-Gorycka, 2022). A meta-analysis by Bae and Lee, (2018) found that RA patients had notably higher levels of circulating miR-146a than the control group. Furthermore, individuals with RA showed higher levels of this marker in synovial tissue/fluid compared to the control group. Additionally, research indicates a positive correlation between miR-146a levels and ESR (Bae and Lee, 2018).

Another microRNA known for its regulatory potential that can impact various immune cells is miR-155 (Doghish et al., 2023; Kmiołek and Paradowska-Gorycka, 2022). In individuals with RA, a positive correlation between miR-155 and inflammation was observed (Kmiołek and Paradowska-Gorycka, 2022). This microRNA plays a role in monocyte recruitment and supports their retention in inflamed areas. Increasing the expression miR-155 in monocytes is linked to decreasing their susceptibility to apoptosis while promoting the production of pro-inflammatory chemokines. Studies suggest that miR-155 maintains T regulatory cell homeostasis (Peng et al., 2023). Researchers propose that the levels of miR-155 are positively associated with ESR and disease activity as measured by DAS28-CRP (Yang et al., 2022).

MicroRNA levels may hold significant potential in predicting the response to treatment for RA (Zhang et al., 2023). Individuals with elevated levels of miR-132-3p, miR-146a-5p, and miR-155-5p in their blood before starting therapy may indicate a likelihood of inadequate response to methotrexate treatment (Peng et al., 2023). Notably, the decrease in miR-132-3p, miR-146a-5p, and miR-155-5p levels induced by methotrexate administration suggests a favorable response to this treatment. A low serum level of miR-15a/16 for therapies involving DMARDs is a prognostic indicator of lower treatment effectiveness. Further research is necessary to explore the role and application of microRNAs in RA (Zhang et al., 2023).

4. CONCLUSION

RA is when the immune system mistakenly attacks the joints, causing long-lasting inflammation. This can result in pain, swelling, and even joint deformities. While it primarily affects the joints, RA can also impact other organs and tissues in the body. CRP and ESR are inflammatory markers often elevated in RA, reflecting the level of inflammation in the body. Proinflammatory cytokines like IL-6 are involved in the development of RA, with elevated IL-6 levels often indicating disease severity. Moreover, this cytokine is a target for biological therapies in RA treatment.

Antibodies in RA include RF, ACPA, and anti-CarP antibodies. Assessment of anti-CarP antibodies may complement RF and ACPA in diagnosing and monitoring the disease. However, RA patients could have anti-CarP antibodies regardless of other markers. MicroRNAs are significant in RA pathogenesis, serving as gene expression regulators involved in inflammation, immune response, and joint damage. Several microRNAs show potential as viable biomarkers for the diagnosis of RA, as well as for predicting outcomes and evaluating treatment responses. Further research is needed to develop precise markers and indicators for monitoring RA treatment.

Author's Contributions

Adrian Kruszewski: Conceptualization, writing - rough preparation, project administration
Natalia Paduszyńska: Conceptualization, supervision
Anna Dąbrowska: Resources, writing - review and editing
Barbara Wawrzyńska: Methodology, writing - rough preparation
Karolina Strus: Conceptualization, data curation
Roksana Zdunek: Methodology, investigation
Magdalena Madera: Formal analysis, visualization

Informed consent

Not applicable.

Ethical approval

Not applicable.

Funding

This study has not received any external funding.

Conflict of interest

The authors declare that there is no conflict of interests.

Data and materials availability

All data sets collected during this study are available upon reasonable request from the corresponding author.

REFERENCES

1. Bae SC, Lee YH. MiR-146a levels in rheumatoid arthritis and their correlation with disease activity: a meta-analysis. Int J Rheum Dis 2018; 21(7):1335-42. doi: 10.1111/1756-185x.13338
2. Carubbi F, Alunno A, Gerli R, Giacomelli R. Post-Translational Modifications of Proteins: Novel Insights in the Autoimmune Response in Rheumatoid Arthritis. Cells 2019; 8 (7):657. doi: 10.3390/cells8070657
3. Catrina A, Krishnamurthy A, Rethi B. Current view on the pathogenic role of anti-citrullinated protein antibodies in rheumatoid arthritis. RMD Open 2021; 7(1):e001228. doi: 10.11 36/rmdopen-2020-001228
4. Doghish AS, Ismail A, El-Mahdy HA, Elkhawaga SY, Elsakka EGE, Mady EA, Elrebehy MA, Khalil MAF, El-Husseiny HM. miRNAs insights into rheumatoid arthritis: Favorable and detrimental aspects of key performers. Life Sci 2023; 314:1213 21. doi: 10.1016/j.lfs.2022.121321
5. Favalli EG. Understanding the Role of Interleukin-6 (IL-6) in the Joint and Beyond: A Comprehensive Review of IL-6 Inhibition for the Management of Rheumatoid Arthritis. Rheumatol Ther 2020; 7(3):473-516. doi: 10.1007/s40744-020-00 219-2
6. Hale AJ, Ricotta DN, Freed JA. Evaluating the Erythrocyte Sedimentation Rate. JAMA 2019; 321(14):1404-1405. doi: 10.10 01/jama.2019.1178
7. Imas JJ, Ruiz-Zamarreño C, Zubiate P, Sanchez-Martín L, Campión J, Matías IR. Optical Biosensors for the Detection of Rheumatoid Arthritis (RA) Biomarkers: A Comprehensive Review. Sensors (Basel) 2020; 20(21):6289. doi: 10.3390/s20216 289
8. Iyengar KP, Vaish A, Nune A. Anti-cyclic citrullinated peptide antibody (ACPA) and Rheumatoid arthritis: Clinical relevance. J Clin Orthop Trauma 2021; 24:101729. doi: 10.1016 /j.jcot.2021.101729
9. Kmiołek T, Paradowska-Gorycka A. miRNAs as Biomarkers and Possible Therapeutic Strategies in Rheumatoid Arthritis. Cells 2022; 11(3):452. doi: 10.3390/cells11030452
10. Kurowska W, Kuca-Warnawin EH, Radzikowska A, Maśliński W. The role of anti-citrullinated protein antibodies (ACPA) in the pathogenesis of rheumatoid arthritis. Cent Eur J Immunol 2017; 42(4):390-398. doi: 10.5114/ceji.2017.72807
11. Kwon EJ, Ju JH. Impact of Posttranslational Modification in Pathogenesis of Rheumatoid Arthritis: Focusing on Citrullination, Carbamylation, and Acetylation. Int J Mol Sci 2021; 22(19):10576. doi: 10.3390/ijms221910576
12. Lapić I, Padoan A, Bozzato D, Plebani M. Erythrocyte Sedimentation Rate and C-Reactive Protein in Acute Inflammation. Am J Clin Pathol 2020; 153(1):14-29. doi: 10.109 3/ajcp/aqz142
13. Lin YJ, Anzaghe M, Schülke S. Update on the Pathomechanism, Diagnosis, and Treatment Options for Rheumatoid Arthritis. Cells 2020; 9(4):880. doi: 10.3390/cells90 40880
14. Matcham F, Scott IC, Rayner L, Hotopf M, Kingsley GH, Norton S, Scott DL, Steer S. The impact of rheumatoid arthritis on quality-of-life assessed using the SF-36: a systematic review and meta-analysis. Semin Arthritis Rheum 2014; 44(2): 123-30. doi: 10.1016/j.semarthrit.2014.05.001
15. Pandolfi F, Franza L, Carusi V, Altamura S, Andriollo G, Nucera E. Interleukin-6 in Rheumatoid Arthritis. Int J Mol Sci 2020; 21(15):5238. doi: 10.3390/ijms21155238
16. Peng X, Wang Q, Li W, Ge G, Peng J, Xu Y, Yang H, Bai J, Geng D. Comprehensive overview of microRNA function in rheumatoid arthritis. Bone Res 2023; 11(1):8. doi: 10.1038/s414 13-023-00244-1
17. Perera J, Delrosso CA, Nerviani A, Pitzalis C. Clinical Phenotypes, Serological Biomarkers, and Synovial Features Defining Seropositive and Seronegative Rheumatoid Arthritis: A Literature Review. Cells 2024; 13(9):743. doi: 10.3390/cells13 090743
18. Pope JE, Choy EH. C-reactive protein and implications in rheumatoid arthritis and associated comorbidities. Semin Arthritis Rheum 2021; 51(1):219-229. doi: 10.1016/j.semarthrit. 2020.11.005
19. Ricchiuti V, Chun KY, Yang JM, Aure MA, Gomez L, Norman GL, Mahler M. Anti-Carbamylated Protein (Anti-CarP) Antibodies in Patients Evaluated for Suspected Rheumatoid Arthritis. Diagnostics (Basel) 2022; 12(7):1661. doi: 10.3390/dia gnostics12071661
20. Scherer HU, Häupl T, Burmester GR. The etiology of rheumatoid arthritis. J Autoimmun 2020; 110:102400. doi: 10.1 016/j.jaut.2019.102400
21. Shapiro SC. Biomarkers in Rheumatoid Arthritis. Cureus 2021; 13(5):e15063. doi: 10.7759/cureus.15063
22. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet 2016; 388(10055):2023-2038. doi: 10.1016/s0140-6736(16) 30173-8
23. Sokolova MV, Schett G, Steffen U. Autoantibodies in Rheumatoid Arthritis: Historical Background and Novel

Findings. Clin Rev Allergy Immunol 2022; 63(2):138-51. doi: 10.10 07/s12016-021-08890-1
24. Takeuchi T, Yoshida H, Tanaka S. Role of interleukin-6 in bone destruction and bone repair in rheumatoid arthritis. Autoimmun Rev 2021; 20(9):102884. doi: 10.1016/j.autrev.202 1.102884
25. Toes R, Pisetsky DS. Pathogenic effector functions of ACPA: Where do we stand? Ann Rheum Dis 2019; 78(6):716-721.
26. Van-Delft MAM, Huizinga TWJ. An overview of autoantibodies in rheumatoid arthritis. J Autoimmun 2020; 11 0:102392. doi: 10.1016/j.jaut.2019.102392
27. Wu CY, Yang HY, Luo SF, Lai JH. From Rheumatoid Factor to Anti-Citrullinated Protein Antibodies and Anti-Carbamylated Protein Antibodies for Diagnosis and Prognosis Prediction in Patients with Rheumatoid Arthritis. Int J Mol Sci 2021; 22(2):6 86. doi: 10.3390/ijms22020686
28. Yang J, Li Z, Wang L, Yun X, Zeng Y, Ng JPL, Lo H, Wang Y, Zhang K, Law BYK, Wong VKW. The role of non-coding RNAs (miRNA and lncRNA) in the clinical management of rheumatoid arthritis. Pharmacol Res 2022; 186:106549. doi: 10. 1016/j.phrs.2022.106549
29. Yoshida Y, Tanaka T. Interleukin 6 and rheumatoid arthritis. Biomed Res Int 2014; 2014:698313. doi: 10.1155/2014/698313
30. Zhang Y, Yang M, Xie H, Hong F, Yang S. Role of miRNAs in Rheumatoid Arthritis Therapy. Cells 2023; 12(13):1749. doi: 10. 3390/cells12131749